Egypt. J. Chem. **Vol. 66,** No. 3 pp. 409 - 419 (2023)

Egyptian Journal of Chemistry
http://ejchem.journals.ekb.eg/

Comparative Phytochemical Screening, Antioxidant, Antimicrobial and Quantitative Analysis of *Pandanus tectorius* and *Rosemarinus officinalis* from Saudi Arabia

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Abstract
1.1. Medicinal plants (MP) have recognized to be a rich source of biologically active compounds for the development of new lead chemicals for pharmaceuticals. Screening of *Pandanus tectorius* and *Rosmarinus offcinalis* plant extracts confirms the presence of various phytochemicals like carbohydrates, cardiac glycosides, coumarin, flavonoids, tannins, terpenoid, steroids and phenols in the selected plants. While other phytochemicals as anthraquinone, alkaloids, saponins, proteins and phytosterol were detected and varied based on the plant constituents. The antibacterial, antifungal and antioxidant activities of the tow plant extracts were established. The antioxidant activity was evaluated by DPPH. The anti-microbial activity determined by the agar well-diffusion method. The results confirm the role of these extracts as promising potent antioxidants and moderate anti-microbial agents. The objective of this study was to determine the phytochemical screening, analysis by LC/MS/MS, the antioxidant, antimicrobial activities of *Pandanus tectorius* and *Rosmarinus offcinalis* ethanolec extract that obtained from AlBaha in Kingdom of Saudi Arabia.
Key words: *Pandanus tectorius* and *Rosmarinus offcinalis*, phytochemical screening and analysis, antioxidant, antimicrobial activities

1. Introduction

The term of medicinal plants (MP) includes a various type of plants utilized in herbalism and approximately of these plants has a therapeutic activity. MP is the "support" of traditional medicine (TM), which means more than 3.3 billion public in the fewer developed nations use MP on a steady source **[1]**. About 80% of people in developing countries use TM for their healthiness. The normal utilization of TM in essential medical care ought to be founded on the rules for the evaluation of homegrown prescriptions as evolved by the World Health Organization (WHO) **[2].**

The natural products that got from MP have shown to be a bountiful wellspring of biologically active compounds, a significant number of which have been the reason for the improvement of new lead synthetic substances for drugs **[3]**. The historical backdrop of TM is basically as old as human civilization. The earliest records affirm that homegrown meds have been utilized and reported in Roman, Greek, Egyptian, Chinese, and Indian restorative frameworks for around 5000 years. MP used in large scale in many countries (developing or primitive) because its biological effects on organisms. Industrial, there are many uses of MP; either it was TM, herbs therapy or health food. The active role of MP lies in existence some active compounds such as: (phenol, flavone, coumarin, alkaloids… etc.) that are considered sources of many drugs in pharmaceuticals. Starting from the beginning of history, plant plays had a significant influence on the treatment of human infirmities. By experimentation, the old populace was easing their enduring by involving spices in an extremely crude manner **[4]**. It is misleading to describe Saudi Arabia "barren desert" when it is there many regions clothed with trees, herbs and flowers as those in the south and some of west area. Most of these plants have effective effect from a medical point of view, but in my research, I highlight aromatic plants that are having biological importance. Examples of these plants, *Pandanus tectorius* (*P. tectorius*) is abundant in south of Saudi Arabia especially in Jizan. There are *Rosmarinus offcinalis* (*R. Offcinalis*) that considered herbs present in the countries surrounding the Mediterranean Sea, in Saudi Arabia. MP assumes a critical part in the turn of events and progression of current examinations of the biological activities of substances **[5]**. Customary medical care frameworks utilizing MP can be perceived and utilized as a beginning stage for the advancement of novelties in

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Receive Date: 26 May 2022, Revise Date: 24 June 2022, Accept Date: 03 July 2022
DOI: 10.21608/EJCHEM.2022.141009.6165

drugs. The controlled utilization of plant substances for prescription is accepted to be less harmful contrasted with those of synthetic products **[6]**.

Pandanus tectorius

In Saudi Arabia, *Pandanus tectorius* (*P. tectorius*) is accounted to be found and comprehensively circulated in the southern districts, especially Jazan governorate **[7]**. *P. tectorius* leaves are narrow and sheathing at the base, thorns are spread across the tips of the leaves. As well, the plant has attractive and aromatic white flowers that belong to flowering plants called "monocot" and grow in warm climates. Biological Active of *P. tectorius*. The biological potencies of (*P. tectorius*) were reported as antiinflammatory, antioxidant, anticancer, antiviral, and antidiabetic and cholesterol lowering activity **[8]**. The primary pieces of *P. tectorius* that are utilized in making customary meds are the natural products, male blossoms, and ethereal roots **[9]**. Pandanus leaves are utilized in therapies for cold/influenza, hepatitis, dysuria, asthma, bubbles, and malignant growth. It is rich by phenolic compounds such as flavonoids, alkaloids such as saponins and terpenes **[10]**.

Rosmarinus offcinalis

Rosmarinus officinalis (*R. officinalis*) is a typical family plant that has a place with the family Lamiaceae and is filled in many areas of the planet precisely in the Mediterranean Sea. *(R. Officinalis*) grows in cold and temperate regions of the Kingdom **[11]**. Usually known as rosemary, is a bush with fragrant, evergreen, needle-like blue, white, purple, or pink flowers and leaves. Biological Active of *R. Offcinalis* Leaves of *R. Officinalis* have an assortment of bioactivities, comprising antioxidant, antitumor, anti- inflammatory, treating cerebral pains and hostile to HIV **[12]**. Rosemary contains flavonoids, phenols, volatile oil and terpenoids **[13]**. The most important constituent of rosemary is caffeic acid and its derivative, Rosmarinus acid **[14]**

Phytochemicals are chemical constituents that produced by plants through primary or secondary metabolism. They are superfluous supplements, implying that they are not needed by the human body for supporting life **[15]**. Alongside these compounds are important to protecting themselves, they have a biological activity of the organism by protective or disease preventive properties and have been used as therapy and others in traditional medicine. The medicinal value of these herbs lies in some phytochemicals witch responsible for biologically active in the human body.

Phytochemical screening alludes to the extraction, overview, and distinguishing proof of the therapeutically active substances found in plants. It is vital in distinguishing new wellsprings of remedially and economically significant compounds like alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins, and terpenoids. Screening of phytochemicals is a significant stage, in the location of the bioactive standards present in restorative plants and in this manner, may prompt medication revelation and improvement.

Classification of phytochemicals

Phytochemicals are basically divided into two groups based on their chemical class, biosynthetic origin and function group **[16]** i.e., secondary and primary ingredients permitting to their purposes in plant uptake as well. The primary elements include communal lipids, proteins, amino acid and sugars. These essential metabolites are significant for the biological functions in plants which incorporate the development, growth, and generation of the plant cell **[17]**. The auxiliary metabolism process in plants produces optional metabolites which are by and large signs of the protection and self-defense in plant cells brought about by the natural lopsidedness or unsafe contaminations **[18]**.Secondary constituents consist of alkaloids, terpenoid **[19]** and polyphenol with all of its derivatives that have biological actives in plants and organisms **[20]**. These secondary metabolites are identical particular and establish boundless in quantities amongst the numerous clusters of plants.

In nature, carbs can be found in practically all living organic entities, remembering for tissues of seeds, stems, and leaves of homegrown plants, the body liquids of creatures, cell walls, and extracellular liquids of microbes, fungi, and yeast **[21]**. Lately, a few bioactive polysaccharides separated and isolated from plants, particularly from the MP, have drawn a lot of consideration in the area of pharmacology and organic chemistry because of their potential biological activities, for example, antitumor, anticoagulant, antioxidant, against diabetic, and immunomodulatory activities. In particular, most polysaccharides got from plants are somewhat non-toxic and don't cause critical incidental effects **[22]**. Numerous polysaccharides and their subordinates have been utilized in an assortment of therapeutic applications.

Plants have antifungal proteins, which not only inhibit the growth of fungi, but are also explored for the treatment of human diseases as production of anticancerous and malarial drugs **[23]**.

Alkaloids are the most important active compounds in herbs. They display antimicrobial and antiparasitic properties. Some alkaloid molecules can act as narcotics which is the painkilling action of morphine. Approximately of them have also importance in the hemoglobinizators of leukemia cells. Furthermore, they performance an identical vital character in the immune systems of organisms and plants. Several alkaloids demonstrate most important biological activities for treating asthma and anticancer activities **[21]**.

Plants containing chemical constituents having a steroidal structure that is responsible for medicinal property as inflammatory disorders such as asthma, rheumatoid arthritis, rhinitis, conjunctivitis, and multiple sclerosis **[24]**. The main utilization of the heart glycosides is its belongings in the treatment of cardiovascular disappointment. On the other hand, some cardiac glycosides used as an antitumor activity and an inhibitory activity against rhinovirus **[25]**.

The present study deals with phytochemical screening, quantitative analysis and biological activities for the leave's extracts of two plants (*P. tectorius* and *R. offcinalis*) that are commonly available in the kingdom of Saudi Arabia.

2. Experimental

2.1. Collection of the herb leaves

Two herbs (*P. tectorius, and R. offcinalis*) were collected from Al-Baha City and the leaves of these herbs were taken and chopped softy.

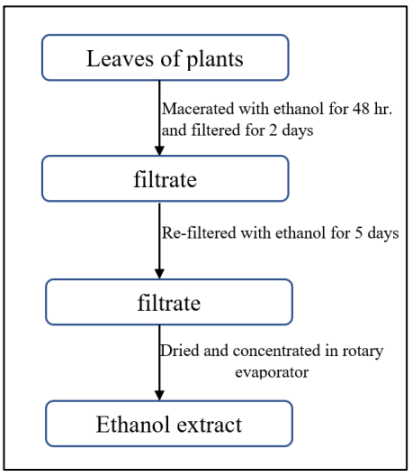
2.2. Herbal Extraction

The two plant leaves were collected and finely grinded after they are dried. Then taken the weights of all the leaves Plants were macerated two times with ethanol (75% concentration) for 48 hr and filtered (**Table 1**).

Table 1: Plants weight and the amount of solvent involved in the Extraction process

No	Plants	Weight (gm)	Solvent (EHOH)	
First time (ml)	second time (ml)		First time (ml)	Second time (ml)
1	*P. tectorius*	300	720	650
2	*R. offcinalis*	225	535	550

The standard extraction scheme used during the study is shown below:



Scheme 1: Filtration process of plants extract

2.3. Phytochemical screening

The extract was prepared by dissolving 0.1gm in 5ml of distilled water and filtrating.

2.3.1.Carbohydrates

Fehling'stest

An equal volume of Fehling solution A and B are added to and equal volume of filtrate and it should boil in a water bath. The formation of red precipitate indicates the presence of sugar **[26]**.

Benedict'stest

A mixture of plant extract and the Benedict reagent is heated a water bath for 2 min and a characteristic-colored precipitate indicates the presence of sugar **[26].**

Molisch'stest

Two ml of the ethanolic extract solution was mixed with 0.2 ml of alcohol solution of α-naphthol (10%) in a test tube and followed with an addition of 2ml of conc.H_2SO_4 by the test tube side. At the interphase of the two layers, a bluish Violet zone is formed that indicates the presence of carbohydrates **[27]**.

2.3.2.Proteins

Biuret test

One drop of 2% CuSO4 solution is added to 2ml of filtrate. Then 1ml of 95% Ethanol is added following by an excess of KOH pellets. Pink color ethanolic layer indicates the presence of proteins **[26].**

Ninhydrin test

Two drops of ninhydrin solution are added to 2ml of the filtrate and purple color proves the presence of amino acids **[26]**.

Xanthoproteic test

The plant extract is treated with a few drops of conc. HNO_3. The formation of yellow color indicates the presence of proteins **[26]**.

2.3.3.Alkaloids

Mayer's test

Two drops of Mayer's reagent are added along the sides of the test tube to few amounts of plant extract. The presence of alkaloid is indicated by a white creamy precipitate **[26]**.

Wagner's test

A few drops of Wagner's reagent are added to a few amounts of plant extract and a reddish brown precipitate depicts the presence of alkaloids **[26]**.

2.3.4.**Steroids (Liebermann-Burchard test)**

In a test tube, 1ml of acetic anhydride was added to 1ml of ethanolic extract, the solution was cooled well in ice followed by the addition of conc. sulphuric acid carefully.

Appearance of color development from violet to blue or bluish-green was an indication for the presence of steroids **[27]**.

b) To 200mg plant extract, add 10ml $CHCl_3$. Take 2ml of this filtrate and add 2ml AC_2O and conc.H_2SO_4. Blue green ring indicates steroids **[28]**.

2.3.5. Cardiac glycosides (Keller-Kiliani test)

In a test tube 2ml of AcOH containing 1-2 drops of 2% solution of $FeCl_3$weremixed with the ethanolic extract and poured into another test tube containing2ml of Conc.H_2SO_4. Brown ring

was formed. It was taken as presented of cardiacglycosides **[27]**.

2.3.6.Phytosterols

Libermann-Burchard's test

2ml of Ac2O was added to 1ml extract and 2ml Conc.H_2SO_4. The color changefrom violet to blue or green indicated the presence of phytosterols **[28]**.

Salkowski's test

The extract is treated with CHCl3 and the filtrate that is treated with a few drops ofAc2O. Then the solution is boiled and cooled and the formation of brown ring at the junction indicates the presence of phytosterols **[26]**.

2.3.7.**Saponins**

The plant extract (1ml) is diluted with distilled water up to 9ml and this is Shaken vigorously for 15 seconds and the extract was allowed to stand for 10 min. The formation of stable thick foam (2cm) indicates the presence of **[26]**.

2.3.8.Terpenoids (Salkowski test)

Approximately 2mL of $CHCl_3$ was mixed with 0.5 gm of the extract. Then, 3mL of conc.H_2SO_4 was added carefully to form a layer. The red color appearance is an indication of terpenoids presence **[27]**.

2.3.9.**Phenols**

The plant extract (2ml) is treated with a few drops of $FeCl_3$ solution in watch glass and the formation of bluish black color proves the presence of phenols **[26]**.

2.3.10. Anthraquinone (Bontrager's test)

Approximately 1gm of the ethanolic extract was placed in a test tube. Then 5ml of benzene was added. It was shaken and filtered. It was followed by an addition of 5ml of 10% NH4OH. The appearance of red, violet or pink color in the ammoniac layer (lower phase) was an indication for the presence of free anthraquinones **[27]**.

2.3.11. **Coumarins**

In a test tube, 1.5ml of extract was mixed with a few drops of NaOH in watch glass. The appearance of yellow Indicated the presence of coumarins **[27]**.

2.3.12. Flavonoids

Alkaline reagent test

A small amount of the extract is treated with a few drops of NaOH and if the intense yellow color solution becomes colorless on the addition of dilute acid proves the presence of flavonoids **[26]**.

Shinoda test

2ml of ethanolic extract was placed in a tube. A few fragments of Mg were added, followed by adding 0.5ml of HCL. The reddish color was an indication of flavonoids presence **[27]**.

2.3.13. **Tannins**

2ml of ethanolic extract was placed in a test tube. Then, drops of 5% FeCl3 were added. A bluish black or greenish coloration was observed. It was an indication of the presence of pyrogallol tannin **[27]**.

2.4. Antimicrobial evaluation

The antimicrobial activity of the plant extract (*P. tectorius, and R. offcinalis* fruits 5g crude extracts in 100 ml of d H_2O) was investigated by well diffusion method. The assay was carried out with four bacterial species: *Escherichia coli* ATCC 25922 (American Type Culture Collection), *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 11778, and two fungal species, *Candida albicans* ATCC 14053 and *Aspergillus niger* isolate. Separately, the six types of target bacteria and fungi suspension (the concentration of the bacteria is 0.5 McFarland standards) were spread evenly by using a cotton swab onto the Mueller Hilton agar (MHA) and broth (Difco Laboratories, Detroit, USA) were used for bacterial growth. Fungal cultures were grown similarly on potato dextrose agar (PDA) and incubated at 27 ˚C for 7 days. One hundred microliters of each plant extracts were placed in petri dish plates. Two antibiotics were used as control, namely tetracycline and cloratzaole. All experiments were performed in triplicate and the plated were incubated at 37 °C for 24 hours for bacterial strains and on 25°C for 48 hours in case of fungal pathogenic test organisms. Clear inhibition zones around the wells indicated the presence of antimicrobial activity. The diameter of inhibition zone (mm) of the crudes and antibiotics was measured and compared [11, 15].

2.5. In Vitro Determination of Antioxidant Activity

2.5.1.Free-radical scavenging activity: DPPH assay

The capacity of the prepared extracts to scavenge the 'stable' free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was monitored according to the method described by Mekni et al. **[29]** with some slight modifications. To 1.0 ml of DPPH (0.3 mm) in ethanol, 2.0 ml of the test samples was added. The reaction mixture was then allowed to stand at room temperature in a dark chamber for 30 minutes. The change in color from deep violet to light yellow was measured at 517 nm in a spectrophotometer (Lambda 265, Perkin Elmer). The free-radical scavenging activities were measured by the decrease in absorbance was then converted to percentage antioxidant activity using the Equation 1:

DPPH scavenging capacity (%) = $[(A_{control} - A_{sample})/(A_{control})]\times 100$

Where $A_{control}$ represents the absorbance of the control and A_{sample} is the absorbance of the test sample.

2.5.2.Determination of total phenolic content

The total phenolic content of all extracts was determined using the Folin-Ciocalteu method described by Mutahar *et al.* **[30]**. Briefly, 0.5 ml of diluted extract was added to a test tube and then mixed with 5 ml of Folin-Ciocalteu reagent (0.2 N). After 8 min, 2 ml of Na_2CO_3 (15%) was added. The reaction

mixture was incubated at 50 °C for 15 min before the absorbance (at 760 nm) of mixtures was recorded against a blank. Total phenolic content of the extracts was calculated from standard gallic acid solutions (0–0.1 mg/ml), and expressed as mg Gallic acid equivalents (GAE) per 100 g fruit dry weight.

$$\frac{(50\ \text{g})\,(0.5\ \text{ml})\,(Y\ \text{g})}{(Z\ \text{g})\,(X\ \text{ml})}.$$

2.6. LC/MS/MS

2.6.1. Conditions and parameters

The analysis of the sample was performed using liquid chromatography–electrospray ionization–tandem mass spectrometry (LC-ESI-MS/MS) with an Exion LC AC system for separation and SCIEX Triple, Quad 5500+ MS/MS system equipped with an electrospray ionization (ESI) for detection.

2.6.2. Positive and negative MRM mode:

The separation was performed using ZORBAX SB-C18 Column (4.6×100 mm, 1.8 µm). The mobile phases consisted of two eluents **A:** 0.1% formic acid in water; **B:** acetonitrile (LC grade)**.** The mobile phase was programmed as follows, 2% B 0-1 min, 2-60% B 1-21 min, 60% B 21-25 min, 2% B from 25.01-28 min. The flow rate was 0.8 ml/min and the injection volume was 3 µl. For Multiple Reaction Monitoring Mass Spectrometry (MRM) analysis of the selected polyphenols, positive and negative ionization modes were applied in the same run with the following parameters: curtain gas: 25 psi; Ion Spray voltage: 4500 and-4500 for positive and negative modes, respectively; source temperature: 400°C; ion source gas 1 & 2 were 55 psi with a declustering potential: 50; collision energy: 25; collision energy spread: 10.

3. Results and Discussion

3.1. Phytochemical Screening

Toward the start of the extraction process, leaves of herbs immersed in ethanol (EtOH) for two days, then were carried out the filtration and preserve the extract. Then immersed the leaves again in EtOH for five days and then filtered. The aim of this method is to try to extract and obtain most of the phytochemicals contained within these leaves to obtain the correct results during screening. According to the references, there are some screenings that have more than one method, such as screening of carbohydrate and proteins has three ways, alkaloid, steroids, phytosterol and flavonoids were screened by two ways. All methods have been carried out in experiments to confirm the apparent results. Phytochemical components of the plants premeditated were examined for the subsequent metabolites: anthraquinone, alkaloid, arbohydrate, cardiac glycosides, coumarin, flavonoid, tannin, saponin, terpenoid, steroid, protein, phytosterol and phenol. The results of phytochemical screening were presented in following (**Table 2**).

Qualitative broadcast consuming ethanolic extract showed the occurrence of greatest the phytochemical ingredients and the absenteeism other of it in plants. As it is seen from the table, the constituents were revealed presenting in varying proportions in plants. Note that, the resulting colors may increase (>+), remain light (+) or absent (-) according to the results observed during the experiment. We also note that, phytochemical that is the most abundant in all plants was **Cardiac glycosides**, as the very dark brown ring appeared on all plants (++ or +++), which can be considered the plants as having an active effect as antitumor activity and an inhibitory activity against. Also from previous results, all phytochemicals present in ***P. tectorius***. The presence of all phytochemicals, especially (cardiac glycosides and phenol) made the plant, promising source of antimicrobial, antioxidant and cytotoxicity properties **[31,32]**.

Despite the presence of all these compounds in the ***P. tectorius***, and by comparing these results with other data obtained by Prakash et al. **[33]** we denoted that, the study denied existence of saponins, coumarins and terpenoids in ***P. tectorius***, as well as another study denied the existence of tannin in this plant **[31]**although these constituents are found in the same plant that were screened in this research. Also, the screening of the presence the alkaloids in ***R. officinalis*** was positive (+), while it did not appear on the same plant in one of the previous studies **[34]**. The difference in the proportion of the presence of phytochemicals and absence it in plants depends on the difference area, weather and soil.

Table 2: Phytochemical screening of ethanol extract of *R.offcinalis* vis *P. tectorius*

Plants	*R. Offcinalis*	*P. tectorius*
Phytoconstituents		
Anthraquinone	-	+
Alkaloid	+	+
Carbohydrate	++	+
Cardiac glycosides	++	+++
Coumarins	++	++
Flavonoid	+	+
Tannins	+	+
Saponins	++	++
Terpenoid	+	++
Steroid	+	+
Proteins	-	+
Phytosterol	-	+
phenols	+	+++

So, these constituents may be absent and appear on the same plant based on these reasons. The Preliminary phytochemical screening of ethanolic extracts (96%) of the plants tabulated in Table 2 showed that *Rosmaris officinalis* contains tannins flavonoids,

terpenoids, alkaloids and carbohydrates but it is free from resins, anthraquinons and saponins. The present results were in agreement with that obtained by Andrade et al., **[8]** as they reported that *R. officinalis* ethanolic extract contains tannins, polyphenol, flavonol ,terpenoid and alkaloid.

3.2. Antimicrobial activity of *P. tectorius* and *R. offcinalis*

The antimicrobial effects of ethanolic extracts of *P.tectorius* and *R. offcinalis* on the growth of various Gram positive, negative bacteria and fungi using the agar well diffusion method are presented in Table 3. The extracts of the selected plants display respectable antibacterial activity alongside fungi and Gram negative as well as Gram positive bacterium. The results indicated that the *P. tectorius* showed a moderate antibacterial activity against all tested pathogens with inhibition zone ranged from 12-14 mm while the extract has a strong inhibitory activity against *S. aureus* with IZ of 18 mm. The obtained results were in agreement to Andriani et al. **[11]**who mentioned that, the maximum antibacterial activities was established ethyl acetate crude extract (PEK) against *E.coli*, *P. aeruginosa*, *S. aureus* and *B. Subtilis* with the range zone of inhibition was 10-15mm, followed by other solvent extracts .

Practically every one of the concentrates showed antibacterial activity against every chosen bacterium. Phytochemical investigation showed the presence of phenolics, flavonoids, steroids, triterpenoids, and saponins, (Table 2) *in P.tectorius* fruits extracts removes, these mixtures could be added to their antibacterial action. Phenolics, flavonoids, and steroids in PEK potentially play a part as antibacterial specialists. Many examinations have revealed phenolics and flavonoids **[35, 36]**, steroidal saponins and triterpenoids saponin **[37]**, and steroidal glycoside **[38]** influenced antibacterial action.

As well as, the results indicated that the *Rosmarinus offcinalis* showed a moderate inhibitory activity against all the tested pathogens with IZ ranged from 12 to 16 mm. The results were in agreement with Weckesser et al. **[39]**, principally against the Gram-positive microbes (*S. aureus* and *B. cereus*). The plant extracts additionally displayed an impact against the Gram-negative microscopic organisms (*E. coli* and *P. aeruginosa*). Nonetheless, this impact was less effective than that introduced against the Gram-positive microbes. A similar behavior was reported by Panizzi et al. **[40]**. With regard to the antifungal activity, the extract inhibits the growth of *C. albicans* with inhibitory activity of 14 mm and 12 mm against *A. niger*. A decent to the moderate antimicrobial action of rosemary medicinal oil has been accounted for by many authors **[41, 42]**.

The *Staphylococcus aureus* was establish to be extra delicate (broadest zones of inhibition) amongst the Gram negative microorganisms (*E.coli* and *P. aeruginosa*), *C.albicans* and *A.niger* were established to be extra resilient as shown in Table (3) in case of both plant extracts. On the other hand, Gram negative microorganisms were extra resistant (a moderate zones of inhibition), than the Gram positive microbes. The Gram-positive bacteria were more sensitive to volatiles extract than the gram-negative bacteria, this observation is in concordance with a previous study reported by Pagliarulo et al. **[43]** who reported that the Gram-positive bacteria were more sensitive to ethanol extracts of volatile compounds of Lamiacae. Meanwhile, Antimicrobial activity is based on *P. tectorius* and *R. offcinalis* species and on extraction efficiency as well as their active compound location. In contrast, our results showed that the ethanol extract of *P.tectorius* and *R. offcinalis* inhibited all the test organisms. This variance may be recognized to locality or seasonal variants.

Table 3: **Phytochemical screening of ethanol extract of *R. offcinalis* vis *P. tectorius***

Plant	Gram +ve bacteria		Gram -ve bacteria		Fungi	
	B.Subtilis	*S. aureus*	*E. coli*	*P. arogenosa*	*C.albicans*	*A.nige.*
P.tectorius	14	18	14	14	14	12
R. ffcinalis	12	16	12	14	12	14

3.3. Antioxidant activity of *P. tectorius* and *R, offcinalis*

3.3.1.Free radical scavenging DPPH assay

A quantitative examination utilizing radical scavenging DPPH measure was trailed by the standard convention of Mekni et al. **[29]**. Quercetin has been utilized as a standard for the cancer prevention agent action of plant extracts. The outcome acquired from the antioxidant activity of the *P. tectorius* and *R. offcinalis* extracts were presented in Table 4 and Figure 1. It shows that the antioxidant activity of both plant extracts was more than 50%.They consider having strong antioxidant activity.

In case of *P. tectorius*

The results obtained in Table 4 were in accordance with Ghasemzadeh et al. **[44]** who revealed that high all-out phenolics and flavonoids content increments antioxidant activity and there was a straight relationship between's phenolics or flavonoids content and antioxidant activity. Related to the phytochemical property in this study, phenolics, flavonoids and steroids, pinoresinol, ethyl caffeate, and different mixtures were significant substance constituents in the *P.tectorius* extract that is answerable for their antioxidant agent action **[45]**. As well as, Englberger

et al. **[46]** mentioned that the *P. tectorius* fruit extract was likewise rich with nutrients like C, E, and β-carotene these all could be correlated to its bioactivities property.

In case of *R. offcinalis*

Rosemary *(R.officinalis)* is a zest and therapeutic spice generally utilized all over the planet. The current study indicated that *the Rosmarinus officinalis* extract has the highest antioxidant activity as shown in Table 4 and Figure 1. These results were in agreement with Peng et al., **[47]** demonstrated that of the antioxidant agents, rosemary has been broadly acknowledged as one of the flavors with the most elevated antioxidant activity. Many mixtures have been disconnected from rosemary, including flavones, diterpenes, steroids, and triterpenes. Of these, the antioxidant activity of rosemary extracts has been basically connected with two phenolic diterpenes: carnosic corrosive and carnosol **[48]**. It is realized that antioxidant activity depends, first, on hereditary and development conditions, like the nature of the first plant, its topographical beginning, the climatic circumstances, reaping date, and capacity and handling, and, second, on the extraction cycle and its chosen boundaries **[49].**

Rosemary extracts got by extraction were demonstrated to be promising concerning their fuse into different food sources, beauty care products, and drug items for which a characteristic smell, variety, and cell reinforcement/ antimicrobial added substance are wanted and to track down potential options in contrast to synthetic additives.

Table 4: Antioxidant Activity DPPH% and Total phenolic compounds of ethanol extract of *R. offcinalis* vis *P. tectorius*

Plant	Antioxidant Activity DPPH%	Total Phenolic Compounds (gGA/1 gextract)
P. tectorius	**81.30**	**06.48**
R. offcinalis	**49.00**	**16.04**
Galic acid	**66.60**	**04.44**

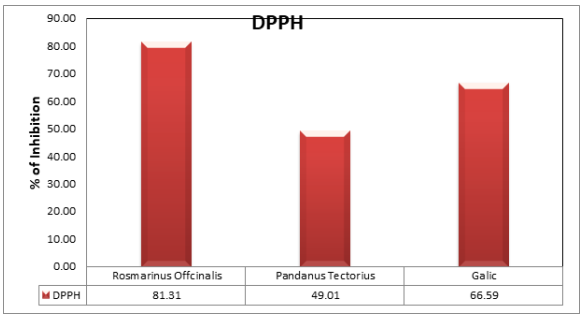


Figure 1. Antioxidant Activity by DPPH% of ethanol extracts *of R. offcinalis vis P. tectorius*

3.3.2.Total phenolic content

The concentration of phenolic in the two plant extracts was presented in Table 4 and Figure 2 was dependent on the solvent and the experimental conditions. The total phenolic content was stated as Galic acid equivalents (GAE) in milligrams per gram of extract. The amount of phenolic compounds in the *P. tectorius* plant ethanol extract was (6.48.mg GAE/1g of extract). These results were lower than the value of 13 mg GAE that obtained from n-hexane extract of stem bark of *P. tectorius* **[32].** The amount of phenolic compounds in the *R. offcinalis* plant ethanol extract (16.4 mg GAE/1g of extract) was the highest. The results were in accordance with Andrade, et al., **[8]** showed that *G. officinalis* extracts showed that gathering of complete phenolic content in various organs was in the scope of 9.13 to 32.76 mg g-1 GAE. In *G. orientalis* extract was distinguished absolute phenolics content from 6.73 to 26.77 mg g-1 GAE. The correspondences between total phenolic contented and antioxidant properties of many plants have been surveyed in earlier studies **[50, 51]**. A few investigations got great positive straight relationships; others acquired poor direct relationships or even couldn't make sense of the correlations between total antioxidant activity and Phenolic content, as introduced by Mata et al. **[52]**, and as happened in our investigation.

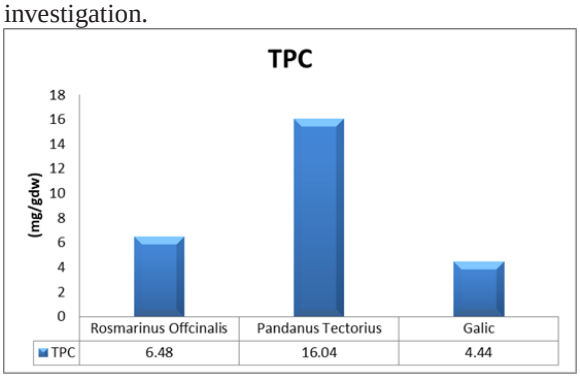


Figure 2 Total phenolic compounds of ethanol extract for *R. offcinalis vis P. tectorius*

3.4. LC/MS/MS Analyses

The ***P. tectorius*** and ***R. offcinalis*** extracts were tested by **LC/MS/MS**. Their composition by µg/ml were shown in Table 5, 6 and Figures 3-4.

P. tectorius

A huge measure of information showed that the sort *Pandanus* contained different classes of secondary metabolites. Thus, roughly 180 constituents were separated, involving amino acids, lipids, steroids, terpenoids, lignans, phenols, alkaloids, lactones coumarins, and flavonoids,. What's more, the critical data associated with food science recommends that Pandanus species can likewise be a decent contender to give natural oils and supplement items in nutrients and sugar **[53]**.The most abundant component in ***P. tectorius*** (µg/ml) were Caffeic acid, l0. 16; lueolin, 1.34; Naringenin, 1.30; 3, 4 dihydroxy benzoic acid,

1.06 (Table 5 and Fig.3). These results were in agreement with Ninh The Son, **[53]** who indicated that numerous extracts from *P. tectorius* stem bark hase been identified, including caffeic acid and p-hydroxybenzoic acid. Ninh The Son, **[53]** affirmed that phenols should have been visible as a character of the tropical plant *P. tectorius*, filling in Vietnam and China. As well as, Liu et al. **[54]** directed a phytochemical overview and showed that a lot of caffeic acid subordinates existed in 75% ethanol extract of Chinese *P. tectorius* dried organic products.

Table 5: LC/MS/MS of *P. tectorius* ethanolic extract

No	Name	Precursor m/z	Rt	Conc. µg/1g extract
1	Chlorogenic acid	355.1/163	7.34	4.25
2	Daidzein	255.1/199	12.93	ND
3	Gallic acid	168.9/124.9	3.85	0.36
4	Caffeic acid	178/135	8.04	203.38
5	Rutin	609/299.9	9.72	0.06
6	Coumaric acid	162.9/119	9.53	7.17
7	Vanillin	151/136	9.57	4.55
8	Naringenin	271/119	15.05	26.01
9	Querectin	301/151	13.59	0.87
10	Ellagic acid	301/145	9.92	ND
11	Hesperetin	301/136	15.64	0.25
12	Myricetin	317/137	11.72	4.25
13	Cinnamic acid	146.9/102.6	14.20	ND
14	Methyl gallate	183/124	7.45	0.36
15	Kaempferol	284.7/93	15.36	0.23
16	Ferulic acid	192.8/133.9	10.25	16.34
17	Syringic acid	196.8/181.9	8.41	4.93
18	Apigenin	269/151	15.05	3.96
19	Catechin	288.8/244.9	7.34	ND
20	Luteolin	284.7/132.9	13.52	2685
21	3.4-Dihydroxybenzoic acid	152.9/109	5.72	21.29

R. offcinalis As a general rule, Rosemary extracts contain numerous bioactive compounds, including phenolic mono-terpenes **[15]**, diterpenes, flavones, and caffeolyl subsidiaries (rosmarinic acid) **[55]**. The results indicated that the most abundant components (µg/ml) in ***R. offcinalis*** were Naringenin 4.25, Caffeic acid, 2.22; apigenin, 0.38; lueolin 0.25 (Table 6 and Fig.4). The obtained results were contracted with **[56]** (Wei; HO, 2006) who reported that the Carnosol is recognized as a main antioxidant in rosemary.

The structure of the rosemary extricates was subjectively like those got by different authors **[40,41,57]**, but with a different quantitative composition. It ought to be noticed that huge varieties in the chemical structure of rosemary extricates have been described. A few elements were found to impact the composition of extracts, including the geographic beginning, part of the plant, the time of reaping, consequently the phenological phase of the plant, and furthermore the extract disengagement technique, natural contrasts inside species, and test extraction time **[58]**

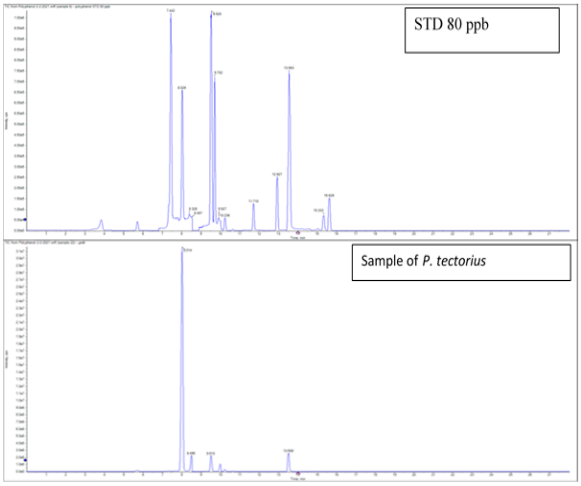


Figure 3: LC/MS/MS of *P. tectorius* ethanolic extract

Table 6: LC/MS/MS of *R. officinalis* ethanolic extract

No	Name	Precursor m/z	Rt	Conc. µg/1gm plant extract
	Chlorogenic acid	355.1/163	7.34	ND
	Daidzein	255.1/199	12.93	ND
	Gallic acid	168.9/124.9	3.85	ND
	Caffeic acid	178/135	8.04	44.42
	Rutin	609/299.9	9.72	0.02
	Coumaric acid	162.9/119	9.53	2.11
	Vanillin	151/136	9.57	ND
	Naringenin	271/119	15.05	85.06
	Querectin	301/151	13.59	0.03
	Ellagic acid	301/145	9.92	ND
	3.4-Dihydroxybenzoic acid	152.9/109	5.72	3.97
	Hesperetin	301/136	15.64	ND
	Myricetin	317/137	11.72	ND
	Cinnamic acid	146.9/102.6	14.20	ND
	Methyl gallate	183/124	7.45	ND
	Kaempferol	284.7/93	15.36	ND
	Ferulic acid	192.8/133.9	10.25	1.07
	Syringic acid	196.8/181.9	8.41	2.46
	Apigenin	269/151	15.05	7.62
	Catechin	288.8/244.9	7.34	ND
	Luteolin	284.7/132.9	13.52	4.98

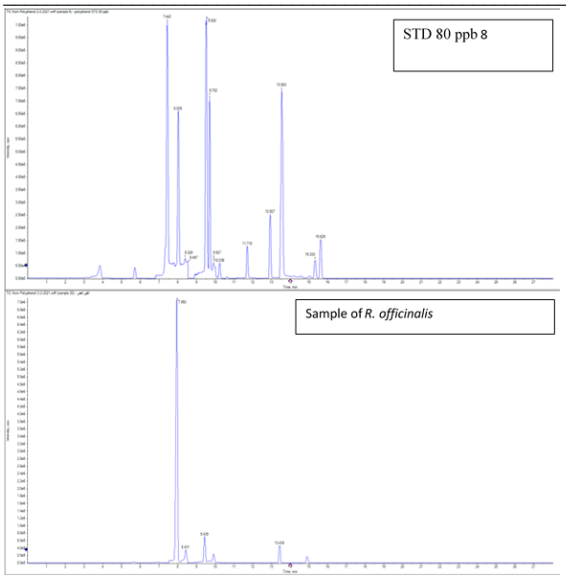


Figure 4: LC/MS/MS of *R. officinalis* ethanolic extract

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